

DRAWINGS ATTACHED

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(54) SPECIFIC CHEMICAL PROBES

(71) We, MILES LABORATORIES INC., a Corporation organized and existing under the Laws of the State of Indiana, United States of America, of 1127, Myrtle Street, Elkhart, Indiana, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to specific chemical probes and to a method of preparing the same.

The science of analytical chemistry has progressed over the past few years with dramatic rapidity. For example, classical analytical chemistry has proved the bio-chemist with test reagents which are highly specific, while modern instrumentation allows a rapid and quantitative readout of the reaction between the compound being detected and the highly specific test reagent. The resulting combination of such specific test reagents and instrumentation has resulted in routine chemical analyses which are precise, sensitive and extremely rapid. Examples of highly specific test reagents are those involving the use of enzymes which react only with specific substrates therefor.

Recently it has been found that specific test reagents such as those utilizing enzymes or substrates therefor can be immobilized or fixed in or on a matrix means such that when the matrix means contacts the fluid or medium being tested, characteristic electrical conductance values and/or a change of electrical conductance values with time (dK/dt) are produced when the fluid contains a specific substance to be detected. When these changes are measured using an electrode system, the values obtained can easily be converted into a quantitative measure of the concentration of a constituent in the fluid being tested. Prior art electrode systems and immobilized or fixed enzyme test apparatus usually comprise two wires held in or on a nonconducting support, the tips of said wires being precisely spaced and enclosed in a matrix means containing

the enzyme, or other specific test reagent. Such specific chemical probes as they are often called are difficult to prepare and reproduce. Probe size is also a critical limitation since the spacing of the wire tips is an important consideration in constructing the device. Moreover, such devices have been found to be extremely flow sensitive, that is, the electrical value is dependent upon the rate of fluid flow past the probe, such as when the solution or fluid being tested is rapidly stirred.

As used hereinafter the term electrode system is used to define one or more electrically conductive elements which sense electrical changes and the term probe or specific chemical probe is used to define the combination of electrode system and matrix containing the specific test reagent.

According to one aspect of the present invention, there is provided a probe for use in detecting a substance in a liquid test medium, comprising an electrically insulating base member, a pair of spaced electrically conductive thin film electrode elements carried on said base member, a semi-permeable matrix means which is insoluble with respect to the liquid test medium and which overlays at least a portion of the electrode elements, and a test reagent which is specifically reactable with said substance and which is contained in said matrix means. The electrode elements, preferably of metal, may preferably be provided with integral electrical contact strips for the facile attachment thereof to a handle and associated electronic circuitry. A preferable form of the present invention comprises the use of a second pair of electrode elements similar to the first but without the test reagent. Such a system provides a differential test apparatus as will be elucidated hereinafter.

A further aspect of the invention provides a method of preparing a chemical probe comprising forming an electrically conductive thin film electrode system on an electrically insulating base member and at least partially overlaying said electrode system with a semipermeable matrix means

containing a test reagent specifically reactable with a substance to be detected.

The invention will be further described, by way of example, with reference to the accompanying drawings wherein:

Figure 1 is an exploded perspective view of one form of apparatus comprising a detachable probe and a handle therefor;

Figure 2 is a diagram showing the various steps involved in making a probe such as that shown in Figure 1; and

Figure 3 is a block diagram of an electronic read out system for use with the probe of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The electrode system of the present invention basically comprises a thin film of a highly conductive material deposited on and bonded to a rigid support or base therefor. The thin metal film is formed into a definite electrode pattern as will be described hereinafter such that the base and film may be used as an analytical specific chemical probe. After forming the electrode elements into a definite pattern, the device is then coated with a semi-permeable matrix means or membrane containing the immobilized test reagent as will be described hereinafter. Strips of the film are extended out from the electrode elements and matrix means such that the device may easily be attached to a handle and/or associated electronic circuitry.

Referring now to the drawings, Figure 1 illustrates a form of apparatus comprising a handle means 21 and a detachable probe 20. The probe 20 is made up of a rigid flat base 10 composed of an insulating material such as ceramic, on one side of which a thin film metal electrode system comprising electrode elements 11 and 22 is "printed" (as will be described hereinafter). End portions of the elements 11 and 22 are formed and positioned to provide the configurations or pattern shown at 19 in which a spaced relationship exists between adjacent end portions of electrode elements 11 and 22. Elements 11 and 22 extend longitudinally along base 10 to form contact strips 23 and 23a for electrical connection to a readout device through cooperating contacts in handle means 21. A fluid insoluble semipermeable matrix means or membrane 12 containing a fixed test reagent is formed over and seals electrode pattern 19 so that when the probe 20 is placed into the fluid to be tested the fluid must first contact the membrane (and test reagent) thus presenting ionic or other chemical changes to the end portions of the elements 11 and 22 at the pattern 19 when the constituent being detected is present in the fluid under analysis. An insulating layer 13 sealingly covers the portions of the elec-

trode elements 11 and 22 between the membrane 12 and the contact portions 23 and 23a prevents false readouts if the probe should be immersed into the fluid being tested somewhat beyond the membrane 12.

Handle means 21 is provided to hold the detachable probe 20 and comprises an elongated pencil-like barrel 14 having a rectangular end opening 15 therein to accommodate and firmly hold the end portion of the probe 20 on which the contact portions 23 and 23a are exposed. Metal spring contact strips (not shown) cooperable with contact portions 23 and 23a are provided within and at one side of the opening 15. Contacts 16 and 16a may be provided within and at the side of the opening 15 opposite the first-mentioned contacts for cooperation with contact portions of a second set of electrode elements (not shown) similar to the elements 11 and 22 fixed to the opposite side of the base 10 in a double-faced version of the probe 20. Handle means 21 also comprises an electrical cable 17 containing insulated electrical wires (not shown) suitably attached at one end to the contacts within the opening 15 and at the other end to a suitable multiple contact connector 18. Ultimately connector 18 is used to facilitate attachment to an electronic readout instrument as will be described hereinafter.

Figure 2 is a diagrammatic illustration of one method which may be used to prepare a probe such as the probe 20 shown in Figure 1. In Step 1, a base member such as a piece of ceramic, glass or plastic about 5 cm. square by 1 mm. thick is thoroughly cleaned, placed in a vacuum metal evaporation system and the system is evacuated.

Step 2 of Figure 2 shows the base member coated with a thin film of a metal such as silver, platinum or gold which may be prepared by known deposition technique such as thermal evaporation by resistive heating or sputtering. The ceramic base member is thus completely coated with a thin metal film of known thickness preferably of from about 1000 Å to 10,000 Å.

The electrode elements shown in Step 3 of Figure 2 may be prepared as follows:

1. The base member previously covered with a continuous thin metal film as described in Step 2 is spin coated with a photoresist such as Shipley Positive Resist No. 1350 and dried at room temperature for 2-3 minutes.

2. A photographic mask (transparent positive) representing the electrode element patterns illustrated in Step 3 of Figure 2 is placed in direct contact with the resist, and the masked base is exposed to ultraviolet light.

3. The latent image formed is developed with conventional techniques and rinsed with water.

4. The base is then placed in an etching solution appropriate for the metal to be removed, such as aqua regia for gold, to remove the metal in the exposed areas, and when etching is complete, the base member is removed and rinsed with water.

5. The etched electrode is then again exposed to ultraviolet light and developed to remove the photoresist from the remaining metal electrodes which may be in the form shown in Step 3 or Figure 2.

The electrodes are then separated into individual units. One method of separation into individual units comprises prescoring the base member and simply snapping the base along the score lines to form the individual units. The result is shown as Step 4 of Figure 2.

A layer of insulation such as an epoxy or silicone resin is applied over the central area of the base member and the electrode elements thereon as shown in Step 5 of Figure 2. The sensing ends of the electrode elements are preferably platinized to improve electrode efficiency, using a 0.3% PtCl in 0.0125 N HCl solution and passing alternating current through the electrode for three minutes at 10 milliamperes/square centimeter.

The end portion of the base member bearing the platinized sensing end portions of the electrode elements is then coated with a test reagent immobilized in a semi-permeable matrix means such as a semipermeable membrane gel as shown in Step 6. The following procedure relates to the preparation of an immobilized urea-determining test reagent in an anisotropic membrane and is merely exemplary:

An organic solvent solution of cellulose derivative and test reagent is prepared by adding 100 mg. of lyophilized crude urease (activity=50 units per mg.) to 20 ml. of acetone and sonicating the mixture for ten seconds. To this mixture is added 1.0 ml. of formamide and 3.0 ml. of cellulose acetate (39.5% acetyl content) and the composite is thoroughly mixed. The end portion of the base member bearing the sensing ends of the spaced electrode elements prepared in Steps 1 to 5 of Figure 2 is then dipped into this solution up to and slightly overlapping the insulating layer overlaying the central portion of the base member and said electrode members removed and air dried for one (1) minute. A second identical dipping and air drying for one (1) minute is followed by a third dip into the cellulose solution, after which the adhering membrane layer is air dried for five (5) minutes. The partially dried membrane layer is then phase inverted by immersing into 0.1 M tris-sulfate buffer, at pH 7.4, for thirty minutes. The result is an anisotropic or structurally polarized membrane

layer coated onto the thin metal film electrode members and the base member.

Figure 3 is a block diagram showing a particularly advantageous differential read-out system for use with the probes of the present invention. Such a system is designed to eliminate background conductivity contributed by the test medium itself. The present invention can readily be adapted to such a differential system by using a probe such as shown in Figure 1 wherein the electrode system comprising elements 11 and 22 is duplicated on the reverse side of base member 10, and the duplicate electrode system is coated with a membrane layer such as the layer 12 but which does not contain the test reagent. In the use of such a probe, the one electrode system comprising elements corresponding to elements 11 and 22 of probe 20 coated with a membrane containing the test reagent senses the electrical characteristics of the test medium plus any changes resulting from the chemical reaction between the substance being detected and the test reagent, while the duplicate electrode system coated with only the membrane simply senses the electrical characteristics of the test medium itself.

More specifically, the apparatus shown in Figure 2 includes a probe 20a comprising a pair of electrode systems 39 and 39a. The electrode systems 39 and 39a, in turn, comprise pairs of spaced electrode elements 11a, 22a and 11b, 22b, respectively. The sensing end portions of elements 11a and 22a are coated with a membrane containing a test reagent, while the sensing end portions of elements 11b and 22b are coated with a membrane which contains no test reagent.

In the circuitry shown in Figure 3 an oscillator 30 is connected to a 6 V. power supply 40 and supplies a voltage to electrode systems 39 and 39a of probe 20a. The input stages 26 and 28, through function switch 24, sense the current flow across the electrode elements 11a, 22a and 11b, 22b and supply D.C. voltages of opposite polarities to the summing amplifier 32, such voltages being proportional to the conductivity at the electrode systems 39 and 39a. The summing amplifier 32, in turn, supplies to the meter circuit 34 a current which is proportional to the difference in conductivity at the electrode systems 39 and 39a. Such differential conductivity is expressed by meter 34 in micromhos. The output current of the summing amplifier 32 is also fed into a differentiator circuit 36 which senses the rate of any change in the output current of the summing amplifier circuit 32 which is also proportional to and indicates the direction of any change in the differential conductivity. Meter circuit 38 indicates changes in differential conductivity in micromhos per second.

A differential system such as that described above is ideally suited for detecting substances in complex biological fluids which have significantly different, as well as continuously changing, electrical characteristics. These electrical characteristics are created by ionic constituents present such as salts, metal ions, and so forth. Exemplary of such biological fluids is blood which is clinically analyzed for substances such as glucose, urea, enzymes, and numerous other complex organic constituents.

The method and apparatus of the present invention may be used for continuous *in vivo* monitoring of patients; and an assembly of several probes as herein described, for detecting a variety of substances, combined with an automated readout mechanism, may be used for the rapid and continuous measurement of a multiplicity of samples for a multiplicity of constituents. Apparatus and probe size is dependent only on state-of-the-art techniques for preparing same and may be dramatically miniaturized for *in vivo* monitoring of biological fluids such as blood for specific substrates as disclosed hereinabove.

It will be appreciated that the materials, methods, structural characteristics, chemical reactions, quantities and so forth as described herein are merely exemplary and may be adjusted to suit the needs for one skilled in the art. For example, the membrane previously described may comprise any of numerous polymeric substances known to those skilled in the art of preparing immobilized test reagents, such as enzymatic test systems. Moreover, electrical characteristics other than conductance may be measured. For example, systems such as those utilizing voltammetry (relationship between current and voltage), E.M.F. (electromotive force) measurements, and so forth may be used. Moreover, other thin film conductive materials such as carbon may be substituted for the metal film described hereinabove.

WHAT WE CLAIM IS:—

1. A probe for use in detecting a substance in a liquid test medium, comprising an electrically insulating base member, a pair of spaced electrically conductive thin film electrode elements carried on said base member, a semi-permeable matrix means which is insoluble with respect to the liquid test medium and which overlays at least a portion of the electrode elements, and a test reagent which is specifically reactable with said substance and which is contained in said matrix means.

2. A probe as claimed in claim 1, wherein the electrode elements are made of metal.

3. A probe as claimed in claim 1 or 2,

wherein the matrix means is a polymeric material.

4. A probe as claimed in any preceding claim, which additionally comprises detachable handle means therefor.

5. A probe as claimed in claim 1, 2, 3 or 4, wherein a second pair of spaced electrically conductive thin film electrode elements is carried by said base member and wherein said second pair of electrode elements is at least partially overlaid by a semi-permeable matrix means which is insoluble with respect to said liquid test medium.

6. A probe as claimed in any one of claims 1 to 4, wherein the spaced electrode elements are connected to a conductivity meter.

7. A probe as claimed in claim 5, wherein the first and second pairs of spaced electrode elements are connected to a differential conductivity meter.

8. A method of preparing a chemical probe comprising forming an electrically conductive thin film electrode system on an electrically insulating base member and at least partially overlaying said electrode system with a semi-permeable matrix means containing a test reagent specifically reactable with a substance to be detected.

9. A method as claimed in claim 8, wherein the thin film is a metal.

10. A method as claimed in claim 8 or 9, wherein said matrix means is a polymeric membrane.

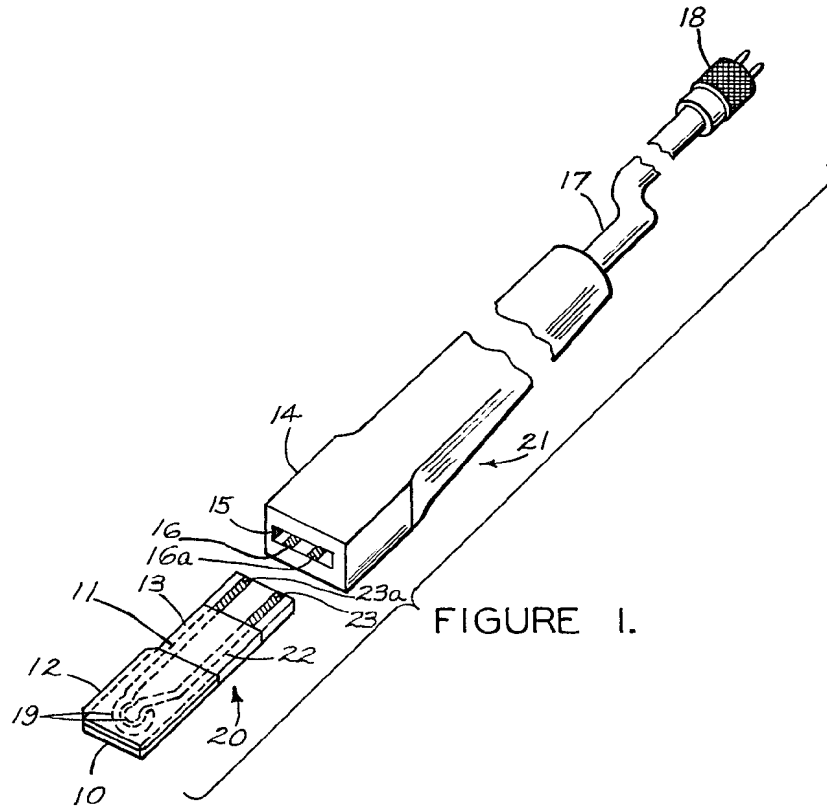
11. A method as claimed in claim 8, 9 or 10, wherein said forming comprises depositing a thin film of highly conductive metal on the base member, applying a photoresist material, exposing the photoresist to a light source through a mask having an electrode configuration, developing and removing portions of the photoresist, chemically etching away the exposed metal and removing the photoresist from the remaining metal film.

12. A method as claimed in claim 8, 9 or 10, wherein said formation is a vapor phase deposition.

13. A method of preparing a chemical probe as claimed in claim 8 substantially as hereinbefore described with reference to the accompanying drawings.

14. Probes as claimed in claim 1 constructed and arranged substantially as herein described with reference to and as illustrated in the accompanying drawings.

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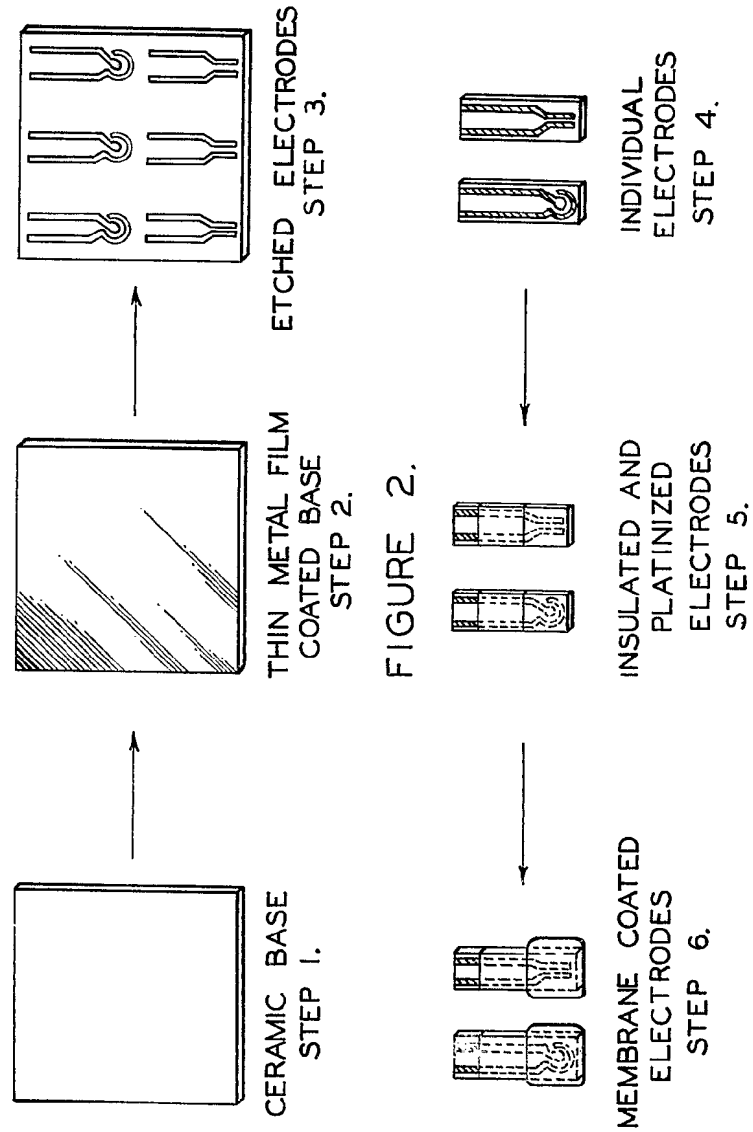


FIGURE 2.

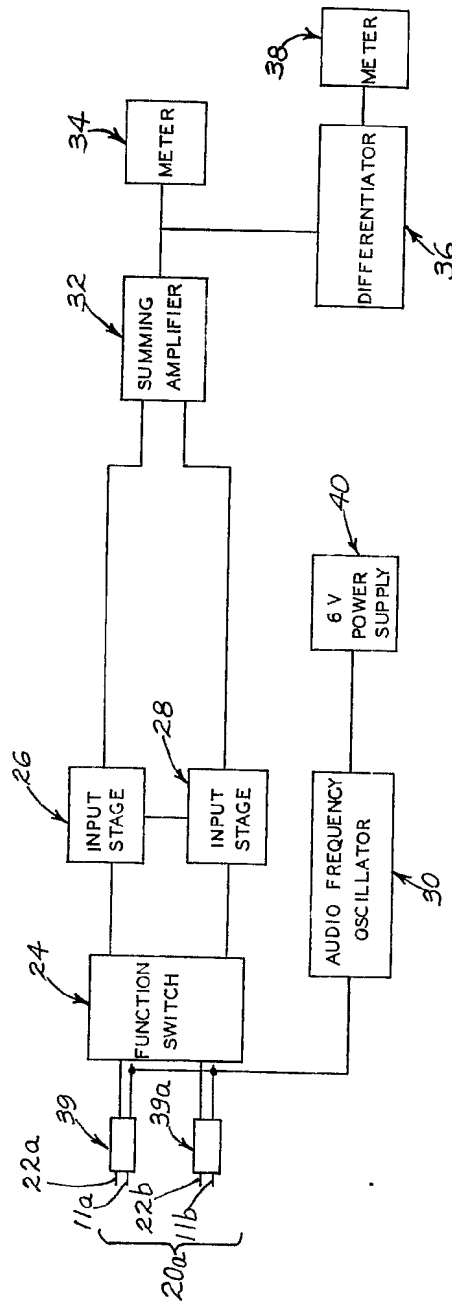


FIGURE 3.